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FIBROSIS MARKERS AND CRIM1 INCREASE IN CHRONIC HEART FAILURE OF INCREASING SEVERITY

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Abstract

Background:The relation between fibrosis biomarkers, suppressors or activators of fibrosis is incompletely studied in CHF of increasing severity.

Aim of the present study is to investigate serum concentrations of fibrosis inductors, suppressors and regulators in patients with CHF of increasing severity and possible correlations between biomarkers and heart functional indexes.

Methods: ELISA tests were used to quantify TGFβ1, TGFβR1,2, FGFa and b, procollagen type(PIP) I, PIP III, Collagen I, III, BMP1,2,3,7, SDF1α, CXCR4, Fibulin 1,2,3, BMPER, CRIM1 and BAMBI in serum of 66 patients with CHF of increasing severity according to New York Heart Association (NYHA class I, n=9; II, n=34; and III n=23), and in 14 healthy subjects matched for age and sex.

Results: TGFβR2, PIP III, SDF1α and CRIM1 were significantly increased in CHF of increasing severity (Kruskal Wallis (KW): p=0.0176, p=0.0021, p=0.0017 and p=0.0332, respectively). In patients with CHF, PIP III correlated significantly with TGFβR2 (r=+0.29, p=0.026) and CRIM1 (r=+0.38, p=0.0052). PIP III and CRIM1 correlated significantly with the six minute walking test (6'WT) (r=-0.37, p=0.008 and r=-0.36, p=0.015, respectively) and with deceleration time (DT-ms) (r=-0.32, p=0.035 and r=-0.43, p=0.010, respectively). Serum levels of all the other molecules studied were similar in CHF and controls.

Conclusions: Fibrosis suppressors such as BMPs are poorly expressed in the serum of CHF patients concomitantly to an increased serum level of the BMPs inhibitor CRIM1, which in turn, is tightly correlated with the serum PIP III increase, suggesting a progressive imbalance in favor of pro-fibrotic mechanisms in CHF of increasing severity.

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Key words

Inflammation, heart fibrosis, endothelial dysfunction.

Introduction

Increased collagen synthesis and degradation has been found in heart failure (1,2) and myocardial fibrosis is associated to development of Heart Failure (3,4). A number of studies performed in postmortem human hearts (5-7) and endomyocardial human biopsies have shown that fibrillar collagen deposition is increased in the myocardium of patients with left ventricular hypertrophy (8-12). Furthermore, fibrosis is higher in more advanced stages of HF and is associated with a poor prognosis (13-17). Serum concentrations of procollagen types (PIP) I and III can be considered as a useful markers of collagens type I and III synthesis (18). Serum collagens I and III concentrations can represent their turnover levels (18). A number of molecules may influence collagen turnover in HF of increasing severity.

TGFβ1 and its receptors R1 and R2 are involved in downregulation of inflammation (19,20), can regulate vascular morphogenesis and extracellular matrix synthesis (21). A pro-angiogenic growth activity and increased cell survival stimulation has been reported for Stromal cell-derived factor-1α (SDF-1α) and its receptor CXCR4 (22,23).

Fibroblasts growth factors (FGF)1 and 2 (acidic and basic, respectively) are involved in the regulation of cardiac angiogenesis and repair (24) and FGF2 is required for development of cardiac hypertrophy (25). Fibulins are involved in structural support, matrix-cell interactions and elastogenic activity (26). Circulating Bone Morphogenic Proteins (BMP) 1-3 may have a pro-fibrotic effect in the kidneys (27). BMP2 can suppresses renal interstitial fibrosis and TGFβ1 induced cardiac fibrosis (28,29). BMP4 reduced TGFβ1 induced extracellular matrix tenascin C and fibronectin production in cultured lung fibroblasts (30). BMP4 inhibitor gremlin is over-expressed in idiopathic pulmonary fibrosis (31). BMP7 suppresses hepatic fibrosis (32), limits Smad3 DNA binding and decreases renal fibrosis (33), reduces collagen content in asbestos treated mice (31), preserves the endothelial cardiac phenotype avoiding the fibroblasts formation and fibrosis (34). BMP endothelial cell precursor derived regulator (BMPER) interacts with BMPs and when overexpressed antagonizes their function (35) or regulates BMPs function (36). Cysteine-rich motor neuron 1 (CRIM1) interacts with BMPs acting as an antagonist reducing production and processing of mature BMPs and decreasing secretion of BMPs (37,38). The BMP and activin membrane-bound inhibitor (BAMBI) is a membrane-spanning glycoprotein that acts as a negative regulator of TGFβ signaling (39). A prevalent anti-fibrotic activity was postulated for the pseudo-

receptor BAMBI that results up-regulated by TGFβ1 stimulation in the endothelium (40).

Aim of the present study is to investigate serum/plasma biomarkers concentrations of fibrosis activators, fibrosis suppressors and regulators in patients with CHF of increasing severity and in a healthy control group of subjects and to evaluate possible correlations between fibrosis regulators and heart functional indexes.

Methods

Subjects

The total study population comprised 80 subjects. The CHF group was composed of 66 subjects with left ventricular dysfunction classified as follows: 9 in NYHA I, 34 in NYHA II and 23 in NYHA III. Characteristics of patients such as mean age, left ventricular ejection fraction (LVEF), sex, 6'WT, VO_2max and etiology (ischemic (IHD) or idiopathic (ICM)) are summarized in tables 1 and 2. Table 2 shows a comparative analysis for age, LVEF%, 6'WT and VO2max between the IHD and ICM subgroups. Patients receiving intravenous infusions, with severe valvular disease or with significant renal insufficiency were excluded from the study. The control group included 14 healthy volunteers matched for age and sex, without any documented history, signs or symptoms of heart failure, nor history of left ventricular dysfunction (table 1). Clinical information, including demographics, co-morbidities, medications, and symptom level based on NYHA classification, were collected in the week before blood sample collection. At enrolment, all subjects underwent complete echocardiographic study. Left ventricular volumes were calculated from orthogonal apical views using the biplane area-length method, and ejection fraction was derived from the standard equation. Deceleration time (DT) of early filling (ms) was measured, as a strong predictor of pulmonary capillary wedge pressure in patients with left ventricular dysfunction (41), irrespective of the degree of mitral regurgitation (42) or atrial fibrillation (43).

The study was carried out in conformity with the Declaration of Helsinki, and informed consent was obtained from each subject. This cross-sectional study was performed according to the local Ethics Committee Guidelines.

Serum collection and analysis

Blood samples were collected using Bekton Dikinson (BD) Vacutainer Cat Plus REF 367896 for serum. Serum or plasma was then aliquoted and immediately frozen at –80

°C until analysis. The serum/plasma levels of all molecules (see table 3) were determined by commercially available enzyme linked immunosorbent assay (ELISA) kits as described in table 3. Table 3 also shows the lower detection limits and the method of quantification (ELISA) for each of the molecules studied. Manufacturers' instructions were carefully followed for each of the ELISA kits used. Further details for each kit are available from the respective online datasheet.

Data analysis

Group data were expressed as mean ± standard error for functional data or median (range or IQR) for serum/plasma levels data. Differences between groups were analysed using analysis of variance (ANOVA) for functional data. The Kruskal Wallis test was applied for serum/plasma levels data, followed - when differences were significant – by the Mann-Whitney U test for comparison between groups. Single regression analysis was performed using the Spearman correlation test. Probability values of $p<0.05$ were considered as significant. Data analysis was performed using the Stat View SE Graphics program (Abacus Concepts Inc., Berkeley, CA-USA).

Results

Subjects

Characteristics of the study population are summarized in Table 1. Patients in NYHA class II and III were slightly older than NYHA I patients. Ischemic patients were slightly older ($p=0.03$) compared to idiopatic subjects and showed a significantly lower VO_2max ($p=0.01$) (see table 2 for characteristics of patients when divided into ischemic and idiopathic subgroups).

Quantification of serum/plasma markers

Quantification of fibroblasts growth factor (FGF) basic and acidic and of Transforming Growth Factor (TGF)β1 were not statistically different in the four groups of subjects studied, showing a low presence of these molecules both in patients and control subjects (table 4). TGFβR1 was slightly decreased in NYHA class II ($p=0.036$) and III ($p=0.020$) compared to controls, even-though this difference was not revealed by Kruskal Wallis test for multiple comparisons. Interestingly, TGFβR2 increased significantly in NYHA class III compared to NYHA class II ($p=0.011$) and controls ($p=0.018$) (figure 1a). The

TGFβ pseudo-receptor BAMBI was similarly expressed in all groups, showing a low expression in the four groups examined. The pro-angiogenic factor SDF1α was significantly increased in NYHA class III compared to NYHA class II (p=0.013), NYHA class I (p=0.014) and controls (p=0.0006) (figure 1b), at variance with its receptor CXCR4, which was similarly expressed in the four groups. Fibulins 1 and 2 were highly expressed in all subjects without any significant differences between groups. Fibulin 4 was significantly higher in NYHA class III patients compared to NYHA I (p=0.011), even though this difference was not confirmed by the more restrictive statistical analysis for multiple comparisons, the Kruskal Wallis test. Among the BMPs (BMP1, BMP2, BMP3, BMP7, BMP10) proteins analysed, BMP1 and BMP2 were the most abundantly expressed in all subjects without significant differences between groups. The BMP endothelial cell precursor derived regulator, BMPER is as well, modestly expressed, at a concentrations similar to BMP2, in all subjects without significant differences between groups. Interestingly, the BMPs antagonist CRIM1, was significantly higher in NYHA class III patients compared to NYHA class II (p=0.024), NYHA class I (p=0.011) and controls (p=0.054) (figure 1c). Collagen I and PIP I were similarly expressed in the four groups of patients studied. Collagen III was significantly higher in NYHA class III patients compared to controls (p=0.043). Most importantly, Procollagen (PIP) type III was significantly higher in NYHA class III patients compared to NYHA class II (p=0.0078), NYHA class I (p=0.047) and controls (p=0.0011) (figure 1d). NYHA class II patients also differed significantly from controls (p=0.038). These results are summarized in table 4.

When ischemic (IHD) were compared to idiopathic (ICM) patients, we did not observe any significant difference for all the molecules studied except for CRIM1 and Fibulin 4 serum concentrations which were reported as significantly higher in IHD compared to ICM patients. These results are summarized in table 5.

Correlations

In our patient cohort, none of the biomarkers studied correlated significantly with LVEF% at echocardiography. PIP III was inversely correlated with 6'WT (r=-0.37, p=0.0086) (figure 2a) and DT (ms) (r=-0.32, p=0.035) (figure 2c). CRIM1 was inversely correlated with 6'WT (r=-0.36, p=0.0158) (figure 2b), peak VO_2 (r=-0.53, p=0.0015) and DT (ms) (r=-0.43, p=0.010) (figure 2d). TGFβR2 was inversely

correlated with DT (ms) (r=-0.35, p=0.027); SDF1α correlated inversely with peak VO_2 (r=-0.42, p=0.032). No other correlations were found between serum/plasma biomarkers and physiological/functional parameters. Interestingly, Pro-collagen type III (PIP III) was positively correlated with TGFβR2 (r=0.29, p=0.026) (figure 3a), CRIM1 (r=0.38, p=0.005) (figure 3b), Fibulin 4 (r=0.35, p=0.010) (figure 3c) and SDF1α (r=0.44, p=0.013) (figure 3d).

Discussion

In our CHF patients, we demonstrated increasing levels of Procollagen type III (PIP III), Transforming growth factor (TGF)βR2, Stromal cell-derived factor(SDF)-1α and Cysteine-rich motor neuron (CRIM)-1, particularly in patients with more severe disease. PIP III serum levels correlated inversely with patient's tolerance to dynamic exercise (6'WT) and to the ventilatory response (DTms). PIP III serum levels were also positively correlated with CRIM-1, SDF-1α, Fibulin-4 and TGFβR2 in the serum of CHF patients of increasing severity. These data, taken together, show a progressive imbalance in favour of pro fibrotic mechanisms, which can be revealed in the serum/plasma, in patients with CHF of increasing severity.

The role of BMPs proteins as anti-fibrotic agents is sufficiently well documented in kidney (44) and liver (32). Relatively few data are disposable in heart fibrosis. Recently, it was showed that in mice, pressure overload induced collagen deposition was decreased and cardiac function improved, after 2 weeks treatment with recombinant BMP-2 (29), suggesting a fibrosis antagonizing action for this molecule. In the serum of our CHF patients BMP-1,-2, -3, -7 and -10 were poorly represented being BMP-1 and BMP-2 the most expressed BMPs proteins. None of the BMPs proteins showed significant changes in CHF patients compared to controls, suggesting that their potential role as anti-fibrotic molecules could not be exerted in CHF patients of increasing severity. Since a number of molecules, such as BMP endothelial cell precursor derived regulator (BMPER) and cysteine-rich motor neuron 1 (CRIM-1) may have a regulatory or inhibitory role on BMPs proteins, we quantified these molecules in the serum of our CHF patients and controls. The BMPER serum levels were relatively low and similar in the four groups of patients studied, suggesting a minor role for this regulatory molecule. Interestingly, we found significantly increased serum levels of CRIM-1 in NYHA class III CHF patients when compared to all the other groups studied and CRIM-1 serum

levels were positively correlated with PIP III serum levels in CHF patients (figure 3). A negative significant correlation was also observed between CRIM-1 serum levels and 6'WT or DT(ms), showing, as an original contribution of the present study, a possible relationship between CRIM-1 levels and increased heart fibrosis, associated to a decreased ventilatory response and dynamic exercise performance of the patients (figure 2). In "in vitro" experiments, CRIM-1 binding of BMP4 and -7 leads to a reduction in the production and secretion of mature BMPs (37) on metanephric explant cultures. These data, together with our "in vivo" findings of an increased serum level of CRIM-1, associated to low serum levels of BMPs, invite to speculate on the possible BMPs inhibitory role of CRIM-1 and subsequent increased heart fibrosis, in our patients with CHF of increasing severity. Interestingly, in the sub-group of CHF patients with ischaemic hearth disease (IHD), CRIM-1 and also Fibulin-4, a molecule involved in the elastic fiber assembly, are significantly increased as compared to idiopathic cardiomyopathy (ICM) group. Also PIP III levels tended to be higher ($p=0.11$) in IHD compared to ICM patients (see table 5), suggesting again a pro-fibrotic role for these proteins in the more compromised patients with CHF.

The TGFβ pseudo-receptor BAMBI, for which a prevalent anti-fibrotic activity was reported (39,45), was similarly expressed in the four groups of subjects studied, suggesting a minor role for this protein in controlling the fibrotic changes developing in CHF of increasing severity.

A pro-angiogenic growth activity has been reported for SDF1α and its receptor CXCR4 (22,23). SDF1α was reported as increased in acute myocardial infarction (AMI) (22), suggesting a contribution of bone marrow cells in myocardial regeneration (22). In CHF patients, a three months physical training significantly increased serum levels of the pro-angiogenic markers angiopoietin-2 and CD34+ cells, but not of SDF1α, as compared with non-trained patients (46). In contrast, a down-regulation of pro-angiogenic mechanisms, included SDF1α reduction, was reported in idiopathic pulmonary fibrosis (47), suggesting that potentially differing mechanisms could be involved in cardiac and lung fibrosis. We here reported a significantly increased serum level of SDF1α in NYHA class III patients compared to all the other groups of subjects and a significant positive correlation between SDF1α and PIP III serum levels in patients with CHF of increasing severity (figures 1 and 3), suggesting a role for this pro-angiogenic molecule in heart remodelling. Since a parallel increase of its receptor CXCR4 was not observed in the plasma of our patients, the effective SDF1α biological

activity developing in these patients needs to be more extensively elucidated by specifically designed “in vitro” studies.

Fibulins have a role in the assembly and stabilization of supramolecular extracellular matrix (ECM) complexes. They also can function as a modulators of cell growth, differentiation and angiogenesis (48). Fibulin-1 and -2 interact with a wide number of ECM complexes, fibulin-4 interacts mainly with tropoelastin (48). Fibulins-1 and -2 were the most expressed fibulins in the serum of our patients without significant differences between groups (table 4). Fibulin-4 was slightly increased in NYHA class III compared to NYHA class I patients, and it was correlated positively with PIP III serum levels (figure 3), probably as a consequence of the more direct relationship of this fibulin type with the cardiac function (48).

Molecules classically considered as fibrosis inductors, such as basic and acidic fibroblast growth factors and transforming growth factor(TGF)β1 were poorly recovered in the serum of our patients and controls (table 4), showing no significant differences between groups. TGFβRI was slightly decreased in NYHA class II and III CHF patients, compared to controls. Interestingly, TGFβRII serum levels, increased significantly in NYHA class III compared to NYHA class II patients and controls (table 4) and correlated significantly with PIP III serum levels (figure 3), showing a potential role in inducing heart remodelling and fibrosis. Pro-collagen (PIP) type I and collagen type I were similarly recovered in the serum of the four groups studied. As expected, PIP III serum level was significantly increased in NYHA class III compared to all the other groups and in NYHA class II compared to controls (table 4). Serum level of Collagen III was also slightly increased in NYHA class III compared to controls (table 4), confirming the increased fibrotic state and collagen turnover in patients with CHF of increasing severity.

The prognostic value of the CRIM-1 serum test, as well as inhibition of the CRIM-1 biological functions and up-regulation of anti-fibrotic BMPs molecules need further specifically planned “in vitro” and “in vivo” studies.

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Figure Legends

Figure 1

Transforming growth factor-β receptor 2 (TGF-βR2) (a), Stromal cell-derived factor-1α (SDF1α) (b), Cysteine-rich motor neuron-1 (CRIM1) (c) Pro-collagen type III (PIP III) (d), in serum/plasma of subjects with chronic heart failure from NYHA class I, II, III and healthy controls. The results are expressed as median (interquartile (IQR) range). Statistical analysis: Kruskal Wallis followed by Mann Whitney U test for comparison between groups. Control subjects: n=14; CHF NYHA class I: n=9; CHF NYHA class II: n=34; CHF NYHA class III: n=23.

Figure 2

Regression analysis between PIP III and CRIM1 serum levels and six minutes walking test (6'WT) (a and b, respectively) and deceleration time (DT-ms) (c and d, respectively) in all patients with CHF (n=66). Spearman's rank correlations.

Figure 3

Regression analysis between PIP III serum levels and pro-fibrotic stimuli TGFβR2 (a), CRIM1 (b), Fibulin 4 (c) and PIP III versus a pro-angiogenic stimulator SDF1α (d) in all patients with CHF (n=66). Spearman's rank correlations.

Tables

Table 1

Characteristics of CHF patients and healthy controls

Subjects (n)	Age	6WT'	LVEF%	VO_2max	Sex (M/F)	IHD/ICM
Controls (14)	53 ± 2	-	60±2	-	14/0	-
NYHA I (9)	55 ± 4	486 ± 23	25 ± 2	20.8 ± 2.3	8/0	4/5
NYHA II (34)	63 ± 2*	405 ± 14	27 ± 1	16 ± 1	31/3	22/12
NYHA III (23)	65 ± 2*	276 ± 31	23 ± 1^	13 ± 1	16/7	13/10

Data expressed as Mean ± SE; IHD = Ischemic Heart Disease; ICM = Idiopathic Cardiomyopathy; LVEF=Left ventricular ejection fraction; 6WT'=six minutes walking test; VO_2max = maximal oxygen consumption.
ANOVA: *p<0.05 from NYHA I; ^p<0.05 from NYHA II.

Table 2

Characteristics of ischemic and idiopathic CHF patients used for analysis of serum/plasma biomarkers

Subjects (n)	Age	LVEF%	6'WT	VO_2max
IHD (39)	65±2	26±1	401±18	15±1
ICM (27)	55±3*	24±1	439±28	20±2**

Data expressed as mean±SE; IHD=Ischemic Heart Disease; ICM=Idiopathic Cardiomyopathy; LVEF =Left ventricular ejection fraction; 6WT = Six minutes walking text; VO_2max = maximal oxygen consumption.
ANOVA: *p=0.03; **p=0.010

Table 3

Summary of the ELISA assays performed in the serum/plasma for biomarkers included in the study

Molecules	Manifacturer (code)	Lower detection limit	Type of plasma/serum	Analytical Method
FGF basic	R&D Systems DFB50	3 pg/ml	Serum	ELISA
FGF acidic	R&D Systems DFA00B	5,68 pg/ml	Serum	ELISA
TGF-β1	R&D Systems DB100B	4,61 pg/ml	Plasma	ELISA
TGF-β R1	USC Life Science Inc. E90397Hu	0,118 ng/ml	Serum	ELISA
TGF- β R2	USC Life Science Inc. E92972Hu	0,126 ng/ml	Serum	ELISA
BAMBI	USC Life Science Inc. E98566Hu	0,064 ng/ml	Serum	ELISA
SDF-1α	R&D Systems DSA00	18 pg/ml	Plasma	ELISA
CXCR4	CUSABIO CSB-E12825h	3.9 pg/ml	Plasma	ELISA
Fibulin 1	USC Life Science Inc. E92472Hu	0,23 ng/ml	Serum	ELISA
Fibulin 2	USC Life Science Inc. E93152Hu	5,6 pg/ml	Serum	ELISA
Fibulin 4	USC Life Science Inc. E95421Hu	1,12 ng/ml	Serum	ELISA
BMP-1	USC Life Science Inc. E90653Hu	0,064 ng/ml	Serum	ELISA
BMP-2	USC Life Science Inc. E90013Hu	15,98 pg/ml	Serum	ELISA
BMP-3	USC Life Science Inc. E92102Hu	27 pg/ml	Serum	ELISA
BMP-7	USC Life Science Inc. E90799Hu	5,9 pg/ml	Serum	ELISA
BMP-10	USC Life Science Inc. E92106Hu	0,066 ng/ml	Serum	ELISA
BMPER	USC Life Science Inc. E98548Hu	0,059 ng/ml	Serum	ELISA
CRIM-1	USC Life Science Inc. E98548Hu	0,13 ng/ml	Serum	ELISA
PIP-I	TaKaRa MK101	10 ng/ml	Serum	ELISA
PIP-III	USC Life Science Inc. E90573Hu	12,8 pg/ml	Serum	ELISA
Collagen I	USC Life Science Inc. E90571Hu	0,237 ng/ml	Serum	ELISA
Collagen III	USC Life Science Inc. E90176Hu	13,1 pg/ml	Serum	ELISA

Table 4

Quantification of pro-fibrotic molecules and growth factors in the serum/plasma of patients with chronic heart failure and control subjects

	Controls	NHYA I	NHYA II	NHYA III	Kruskal-Wallis P- value
FGF basic (pg/ml)	0(0-0.33)	0 (0-1.67)	0(0-9.07)	0(0-4.02)	0.4240
FGF acidic (pg/ml)	0(0-38.07)	0(0-14.78)	0(0-468.41)	0(0-22.52)	0.2608
TGF-β1 (ng/ml)	0(0-25.41)	0(0-8.6)	0(0-16.98)	0(0-18.18)	0.9657
TGF-β R1 (ng/ml)	1.04(0.36-5.76)	0.84(0.01-20.84)	0.6(0-5.96) ≠	0.44(0-4.2) ≠	0.0724
TGF- β R2 (ng/mL)	1.00(0-26.99)	2.37(1.13-23.61)	1.49(0-26.162)	4.29(0.476-32.784) ≠∇	**0.0176**
BAMBI (ng/mL)	0.24(0.18-1.11)	0.26(0.11-10.09)	0.22(0.07-1.24)	0.29(0.03-0.81)	0.3363
SDF-1α (pg/mL)	1853(1440-2179)	2035 (1743-3123)	2035 (1447-3604)	3088 (1736-4004) ≠ε∇	**0.0017**
CXCR4 (pg/ml)	59(13-109)	19(14-103)	46(7-213)	47(1-163)	0.6997
Fibulin 1 (ng/ml)	36700(16400-110500)	37650(17400-99400)	35000(16400-139200)	25700(14400-108500)	0.3272
Fibulin 2 (ng/ml)	615(141-2442)	180(123-2189)	881(105-2664)	1165(126-2455)	0.9345
Fibulin 4 (ng/mL)	14.04(2.27-30.66)	7.52(2.50-14.27)	11.22(0.68-203.40)	16.27(5.91-60.60) ε	0.0651
BMP-1 (ng/mL)	0.94(0.56-10.95)	1.50(0.80-10.40)	1.52(0.57-16.87)	2.03(0.84-9.45)	0.2645
BMP-2 (pg/mL)	424(206-820)	457(146-4760)	500(25-2328)	288(109-1376)	0.8584
BMP-3 (pg/mL)	0(0-6782)	0(0-4981)	0(0-5331)	0(0-5043)	0.7661
BMP-7 (pg/mL)	0(0-503)	0(0-429)	0(0-530)	0(0-105)	0.8054
BMP-10 (ng/ml)	0.02(0-1.8)	0(0-8.8)	0(0-1.47)	0(0-1.8)	0.4457
BMPER (ng/ml)	0.50(0.39-2.93)	0.47(0.26-13.7)	0.46(0.22-2.02)	0.50(0.22-3.95)	0.5758
CRIM-1 (ng/mL)	1.18(0-5.23)	0.75(0-1.79)	1.16(0-26.10)	2.67(0-26.50) ≠ε∇	**0.0332**
PIP-I (ng/mL)	574(335-1417)	566(209-936)	450(198-1310)	556(267-1421)	0.2589
PIP-III (ng/mL)	48(20-87)	50(29-159)	67(28-147) ≠	106(25-262) ≠ε∇	**0.0021**
Collagen I (ng/ml)	330(169-1000)	278(75-1000)	292(76-1000)	337(89-1000)	0.6347
Collagen III (ng/ml)	5198(0-15062)	8083(172-22428)	5458(0-30840)	9400(0-64386) ≠	0.2169

Result expressed as median (range)

Mann-Whitney U test: ≠ significantly different from controls p<0.05; ε significantly different from NHYA I p<0.05; ∇ significantly different from NHYA II p<0.05.

Table 5. Median values of biomarkers in the serum/plasma of chronic heart failure patients according to ischaemic or idiopathic aetiology of the disease

Subjects (n)	CRIM 1 (ng/mL)	Fibulin 4 (ng/mL)	PIP III (ng/mL)	Collagen III (ng/mL)
IHD (39)	2.2 (0-26.49)	15 (1.3-60)	83356 (26566-246472)	8168 (172-64386)
ICM (27)	0.74 (0-2.17)	8.1 (0.68-16.62)	53302 (27600-172952)	8261 (0-31950)
p-value	0.0102	0.0106	0.115	0.463

Tabella formattata

Values are expressed as median (range). ICM, idiopathic cardiomyopathy; IHD, ischaemic hearth disease; CRIM1, Cysteine-rich motor neuron-1; PIP III, Pro-collagen type III. Mann-Whitney U-test for comparison between groups.